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CORTICOSTERONE BINDING GLOBULIN: AN ACUTE PHASE "NEGATIVE" PROTEIN IN THE RAT

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1. Introduction

We report a novel effect of an induced inflammatory process on the serum proteins of the rat: the acute phase of the inflammation is accompanied by a dramatic decrease of corticosterone-binding globulin (CBG or transcortin) activities in the normal and the 19-day pregnant adults; at the same time, the exceptionally high fetal CBG activities of this pregnancy stage [1] are not affected. Analysis of binding parameters reveals that the observed variations are due to a fall in the number of binding sites, whereas the affinity of the protein for the hormone is not significantly modified.

This is, to our knowledge, the first report on the implication of a specific high affinity serum hormone carrier in an inflammatory reaction.

2. Methods

2.1. *Animals*

Sprague–Dawley (Charles River) 2-month-old male and female (17-day pregnant and non-pregnant) rats were used. Inflammation was induced by a single subcutaneous turpentine injection (0.5 ml/100 g), always given at 5.00 p.m. The animals were killed 40 h later, i.e., in the case of the pregnant rats, on the 19th day of gestation. The timing corresponds to a maximum increase of the acute phase serum proteins in these experimental conditions (Lombart, C., personal communication).

Blood was collected by means of a catheter in the abdominal aorta of the adults and after decapitation of the embryos. Sera were prepared separately from individual adults and the different embryo pools. Control sera were obtained from the same categories of untreated animals.

2.2. *Steroids*

1,2,6,7[^{3}H]corticosterone, 81 Ci/mmol (Radiochemical Centre, Amersham); 2,4,6,7(n)[^{3}H]estradiol-17β, 87 Ci/mmol (Radiochemical Centre, Amersham). Radioinert corticosterone was purchased from Roussel-Uclaf.

2.3. *Binding studies*

We have applied a batchwise equilibrium dialysis technique, with a suspension of Sephadex G-25 as the semi-permeable membrane [2]. Some experiments were carried out in parallel by non-equilibrium gel filtration on Sephadex G-50 columns. Stripping of endogenous steroids was performed either by addition of charcoal or by gel filtration at 45°C [3]. Proteins were assayed according to Lowry et al. [4].

The binding data were analyzed by the graphic methods of Pearlman and Crépy [2], Scatchard [5] and Rosenthal [6]. We have measured the following binding parameters: "1/P" indexes (L/g), where P is the protein concentration corresponding to an equilibrium ratio of steroid unbound/steroid bound =1; association constants (K_a); concentration of binding sites n_1 X mol of binding protein M_1 ($n_1M_1 \cdot g^{-1}$ of serum proteins).

2.4. *Electrophoretic studies*

Analytical electrophoresis in 7% polyacrylamide gels was performed on sera preincubated overnight at 4°C with tritiated corticosterone ($2 \cdot 10^5$ cpm/100 g/ml serum proteins) essentially as described [7]. The

radioactive patterns of the electropherograms were compared to Coomassie Blue stained patterns of gels run in parallel with the corresponding non-labelled sera.

2.5. *Haptoglobin assay*

As an index of the inflammatory response we have used the increase of serum haptoglobin [8,9]. This protein was measured in several sera studied by immunonephelometry [10].

3. Results

3.1. *Binding of corticosterone by whole sera from inflammatory and control rats: gel equilibration and gel filtration studies*

Table 1 presents the corticosterone binding values measured at equilibrium by gel dialysis on sera from normal and inflammatory rats of different age and physiological condition. Data on the haptoglobin content of some of these sera are also given. Fig.1 illustrates a gel filtration experiment on the interaction of corticosterone with the serum proteins from a stressed and a control adult male.

It may be seen that the binding of the steroid is significantly decreased in inflammatory non-pregnant and decreased in inflammatory pregnant adults, compared to the corresponding untreated controls. The concomitant 6–8-fold increase of haptoglobin is also manifest, indicating that the negative binding response to the stress is coincident with the opposite variation of this well studied acute phase serum reactant.

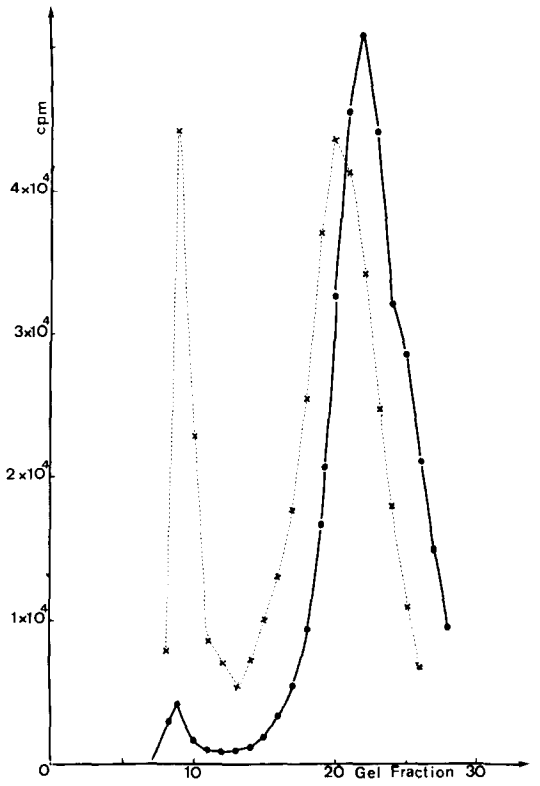

Fig.1. Gel filtration of sera from an adult normal male (X···X) and an adult inflammatory male (●——●). After removal of endogenous hormones, the sera were incubated 3 h at 37°C with tritiated corticosterone (0.3 ml serum/1 μCi [^{3}H]corticosterone), and applied to Sephadex G-50 columns (10 × 0.8 cm). Elution was performed at 4°C with a phosphate buffer, 0.5 M pH 7.4. The excluded peaks correspond to the amount of bound hormone.

Table 1
"1/P" corticosterone binding indexes (l/g) and haptoglobin concentrations (g/l) in inflammatory and normal control rat sera

Origin of serum	Inflammatory		Control		p[a]
	1/P	Haptoglobin[b]	1/P	Haptoglobin[b]	
Adult male	3.3 ± 2.0	–	11.2 ± 2.5	–	<0.001
Adult female	5.5 ± 1.5	1.8 ± 0.2	13.4 ± 0.5	0.19 ± 0.01	<0.001
19-day pregnant	6.4 ± 2.5	1.3 ± 0.2	9.7 ± 3	0.21 ± 0.02	<0.01
19-day embryo	23.5 ± 4	–	22.6 ± 4.5	–	NS

[a] Significance of difference of inflammatory vs. control groups as regards "1/P" corticosterone binding values (Student's *t* test)

[b] Means of 3–4 determinations ± SE

NS, Non-significant

Data are expressed as means of 6–8 determinations ± SE

Table 2
"1/P" estradiol binding indexes (1/g) in fetal rat sera from inflammatory and control normal mothers

Inflammatory	Control	p[a]
192 ± 2	195 ± 1	NS

[a] Significance of difference of inflammatory vs, control groups

Data are expressed as means of 4 determinations ± SE

By contrast, the fixation of hormone in the embryos does not appear affected by the stress condition of the mother. Neither is the elevated estrophilic ability of the α_1-fetoprotein modified in fetuses from turpentine-treated females (table 2).

3.2. *Electrophoretic studies*

To ascertain that the electrophoretic behavior of the corticosterone-binding protein(s) is not altered as a result of the inflammatory process and also as additional evidence for the decreasing effect of this process on hormone fixation, we have analyzed the radioactive and stained patterns of gel electropherograms run in parallel with control and inflammatory serum proteins. Fig.2 presents an electrophoresis experiment with 19-day-pregnancy sera. A single peak of radioactivity located at the level of the α_1-globulins, i.e.

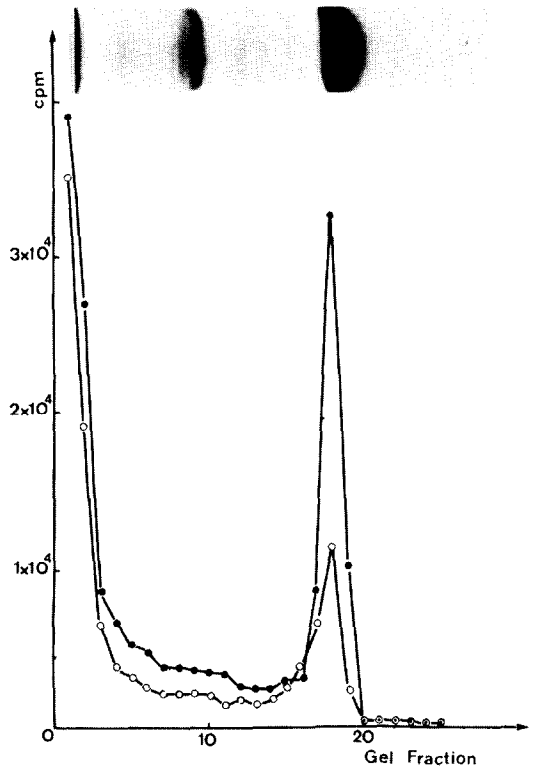

Fig.2. Electrophoresis of [^{3}H]corticosterone pre-labelled sera from a turpentine-treated 19-day pregnant (○——○) and a control 19-day pregnant (●——●) rat.

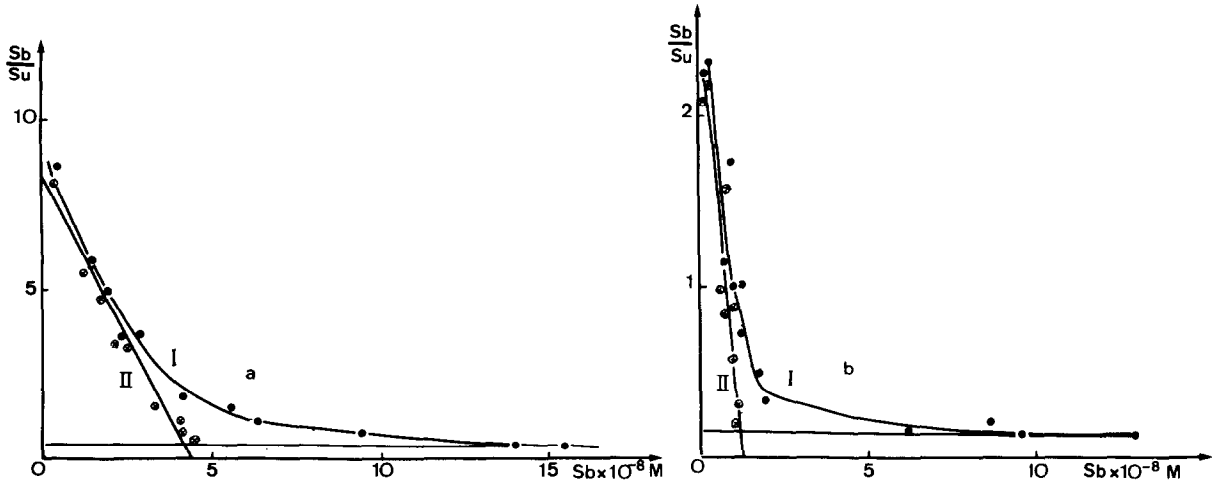

Fig.3. Scatchard plots and Rosenthal corrections for the binding of corticosterone to a control (a) and an inflammatory serum (b) from non-pregnant females. The reaction mixtures consisted of 200 mg Sephadex G-25 "fine", 1.27 (a) 1.14 (b) mg serum proteins, 0.5–600 ng corticosterone, in 2 ml H_2KPO_4/HNa_2PO_4 buffer 0.15 M pH 7.4. 1 h dialysis at 25°C under agitation. Each point represents the mean value of a triplicate assay. Curve I: Experimental data. Curve II: Correction according to Rosenthal. (a) K_a = $1.9 \cdot 10^8\ M^{-1}$; $n_1M_1 \cdot g^{-1}$ serum proteins = $4.9 \cdot 10^{-8}$ M. (b) $K_a = 1.9 \cdot 10^8\ M^{-1}$; $n_1M_1 \cdot g^{-1}$ serum proteins = $1.5 \cdot 10^{-8}$ M.

Table 3
Binding parameters for the interaction of corticosterone with sera from control and inflammatory rats

Serum	$K_a\ 10^8 \cdot M^{-1}$	n_1M_1/g serum proteins $\cdot\ 10^{-8}$
Control non-pregnant	2.40	2.80
Treated non-pregnant	2.70	0.82
Control pregnant	2.80	2.50
Treated pregnant	2.30	0.98

Means of 3 determinations. Experimental conditions as in legend of Fig.3

with the electrophoretic characteristics of CBG, is seen in both turpentine-treated and untreated animals. A 3-fold diminution of the radioactivity associated with this globulin is manifest in inflammatory serum. Similar results (not shown) were obtained with non-pregnancy adult plasma proteins.

3.3. *Scatchard analysis*

Fig.3 presents typical Scatchard plots for the interactions of corticosterone with a non-pregnant female serum (a) and with the serum from a non-pregnant female undergoing acute inflammation (b). Mean values of association constants and concentrations of binding sites are given in table 3. A single major class of high affinity saturable binding sites appears to be present in all cases. Association constants display very similar values for all the tested animals, whether treated or untreated. By contrast an average three-fold decrease of the $n_1M_1 \cdot g^{-1}$ values, characteristic of the binding capacity, is manifest in the treated vs. control rats.

Thus the fall of the corticosterone binding under the effect of inflammation is best explained by a serum CBG depletion, rather than by the alteration of the intrinsic affinity properties of the binding protein.

4. Conclusion and discussion

Acute inflammation induced by an s.c. turpentine injection provokes an average 3-fold decrease of the serum corticosterone binding in adult rats, whether pregnant or non-pregnant. By contrast, the corticosterone fixation in fetal sera is not affected, in our conditions, by the inflammatory status of the mothers. The fall of these activities is associated with an ~3-fold reduction of serum concentrations of the high affinity binding sites characteristic of transcortin (CBG) and involves no significant modification of the affinity properties or electrophoretic behaviour of this binding protein.

At least ten serum proteins are known to increase during the acute phase of inflammation [11,12], whereas very few serum proteins are known to decline under this stress. Along with that of transferrin and albumin [9,12], the CBG reduction documented here adds a novel feature to the as yet scanty picture of "negative" acute phase proteins. Provided that a similar variation is proved in man, it might lead to an easy and reliable test for clinical evaluations.

As regards the mechanism of the CBG decrease, our data would suggest a change of the serum levels of the protein rather than of its physicochemical properties. It remains to be clarified whether this involves an accelerated catabolism, a transfer to extravascular spaces, an inhibited hepatic synthesis or secretion.

Our results are also relevant to the problems of function and control of serum hormone binders, particularly of CBG. Extensive work from this and other laboratories has shown the complexity of the implied factors: species, sex, age, physiological and pathological conditions, experimental manipulations [1,7,14–16]. The rat is unique in that the CBG activities of the 19-day fetus largely surpass those of the mother, suggesting the possible existence of a specific "fetal" transcortin or alternatively a privileged transfer of the protein from mother to embryo [1]. Comparisons of embryo and maternal CBG variations under definite circumstances, like the inflammatory stress explored here, seems a promising approach in this problem. However, in order to draw any conclusion on this point from the independent inflammatory responses of the maternal and fetal CBGS, detailed time-course comparative studies are required.

It is interesting to correlate the inflammatory CBG reduction with the corticosterone hypersecretion evidenced by other authors in similar conditions [13]. These effects may be compared to the fall of CBG brought about by corticosterone administration in adrenalectomized rats [16]: they provide additional evidence for an endocrine control of the serum transcortin levels. It is tempting to speculate that the decline of CBG, by increasing the unbound fraction of circulating glucocorticoids, enhances the anti-inflammatory action of hormones.

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